Free Radical Biology & Medicine 50 (2011) 1669–1678

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Nectarine promotes longevity in *Drosophila melanogaster*

Olga Boyd [a,1], Peter Weng [a,1], Xiaoping Sun [a], Thomas Alberico [a], Mara Laslo [a], David M. Obenland [b], Bradley Kern [a], Sige Zou [a,*]

[a] *Laboratory of Experimental Gerontology, National Institute on Aging, Baltimore, MD 21224, USA*
[b] *Agriculture Research Service, U.S. Department of Agriculture, Parlier, CA 93648, USA*

ARTICLE INFO

Article history:
Received 21 October 2010
Revised 7 March 2011
Accepted 8 March 2011
Available online 13 March 2011

Keywords:
Aging
Nectarine
Peach
Life span
Oxidative stress
Reproductive aging
JNK signaling pathway
Nutraceutical
4-Hydroxynonenal–protein adduct
Free radicals

ABSTRACT

Fruits containing high antioxidant capacities and other bioactivities are ideal for promoting longevity and health span. However, few fruits are known to improve the survival and health span in animals, let alone the underlying mechanisms. Here we investigate the effects of nectarine, a globally consumed fruit, on life span and health span in *Drosophila melanogaster*. Wild-type flies were fed standard, dietary restriction (DR), or high-fat diet supplemented with 0–4% nectarine extract. We measured life span, food intake, locomotor activity, fecundity, gene expression changes, and oxidative damage indicated by the level of 4-hydroxynonenal–protein adduct in these flies. We also measured life span, locomotor activity, and oxidative damage in *sod1* mutant flies on the standard diet supplemented with 0–4% nectarine. Supplementation with 4% nectarine extended life span, increased fecundity, and decreased expression of some metabolic genes, including a key gluconeogenesis gene, PEPCK, and oxidative stress-response genes, including peroxiredoxins, in female wild-type flies fed the standard, DR, or high-fat diet. Nectarine reduced oxidative damage in wild-type females fed the high-fat diet. Moreover, nectarine improved the survival of and reduced oxidative damage in female *sod1* mutant flies. Together, these findings suggest that nectarine promotes longevity and health span partly by modulating glucose metabolism and reducing oxidative damage.

Published by Elsevier Inc.

Aging is a multifaceted process associated with a gradual decline in physiological function and an increased incidence of various diseases, including cancer, neurodegenerative diseases, and diabetes [1,2]. Increasing evidence has demonstrated that many fruits and their extracts contain high levels of phytochemicals, and fruit consumption promotes health and prevents or delays age-related diseases [3–6]. However, few preclinical studies in animals have emerged demonstrating that specific fruits when supplemented in the diet promote longevity and health span.

Numerous genetic and environmental factors have been implicated in modulating aging processes [1,2]. Among many hypotheses of aging, the free radical hypothesis proposed more than 50 years ago states that cumulative oxidative damage to macromolecules in the cell is a causal factor of aging [7]. Since then, numerous lines of evidence have been discovered to support this hypothesis but also point out that the effect of oxidative stress on life span depends on the environmental context [8,9]. Genetic studies have identified hundreds of genes that are involved in modulating life span in yeast, worms, flies, and rodents. These genes can be categorized into a number of signaling pathways, including the insulin/insulin-like, target of rapamycin (TOR)[2], Jun kinase (JNK), and sirtuin signaling pathways, which are functionally conserved across diverse species [1,2,10,11]. Single-nucleotide polymorphisms in some longevity-associated genes, such as insulin-like growth factor receptor, have been linked to human longevity because they are enriched in centenarians [12–15]. Some of the longevity-associated pathways, such as the JNK signaling pathway, mediate oxidative stress response in the cell. This is consistent with the free radical hypothesis of aging, although increasing resistance to oxidative stress does not necessarily result in longer life span.

Food consumption, an environmental factor, plays a pivotal role in modulating the life span of an organism. Dietary restriction (DR), a potent environmental intervention, can extend the life span and health span in various species, although DR does not always promote longevity [1,16–18]. DR is also effective in reducing the incidence of cancer, diabetes, and other diseases in mammals. Life span extension by DR may be mediated through several nonexclusive genetic pathways, such as the sirtuin, SKN-1, and TOR pathways [1,2,10].

Another prolongevity intervention is to supplement the regular diet with health-promoting nutraceutical compounds and extracts derived from plants and fruits. A prominent feature of nutraceutical extracts is that they have high levels of polyphenols, which possess

Abbreviations: DR, dietary restriction; *sod1*, superoxide dismutase 1; TOR, target of rapamycin; JNK, Jun kinase; 8-oxoG, 8-oxoguanine; SY, sugar–yeast extract; RNAi, RNA interference; UAS-*sod1IR*, UAS-*sod1* inverted repeat; da-Gal4, daughterless-Gal4; DAMS, *Drosophila* Activity Monitor System; qPCR, quantitative polymerase chain reaction; 4-HNE, 4-hydroxynonenal; PEPCK, phosphoenolpyruvate carboxykinase; Prx, peroxiredoxin.

* Corresponding author. Fax: +1 410 558 8302.
E-mail address: zous@mail.nih.gov (S. Zou).

[1] These authors contributed equally to this project.

0891-5849/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.freeradbiomed.2011.03.011

high antioxidant activities and other health-promoting properties [4,19]. Polyphenols have been shown to reduce the incidence of various types of cancer, type 2 diabetes, and other diseases [4,19]. Several nutraceutical compounds and extracts have been shown to promote longevity in diverse species. Resveratrol, a polyphenol compound, can extend life span in yeast, worms, flies, and fishes on their respective regular diets, and mice on a high-fat diet [20–23], through activating several longevity-associated genes, including sirtuins [24]. Blueberry extract has been shown to extend the life span of *Caenorhabditis elegans* partly through an osmotic stress resistance-related Ca^{2+}/calmodulin-dependent protein kinase pathway [25]. Depending on the composition of diets, a mixture of cranberry and oregano extracts can extend life span without compromising fecundity in Mexican fruit flies [26]. Pulp extract of açai, a berry indigenous to the Amazon River region, can improve survival of flies on a high-fat diet partly through activating the JNK pathway and reducing expression of genes in gluconeogenesis [27]. However, the number of prolongevity nutraceutical compounds and extracts identified so far is still small. In some cases, their effectiveness in promoting longevity has been challenged. Some other studies in yeast, worms, and flies have shown that no or marginal life span extension was induced by resveratrol [28,29]. Moreover, the mechanisms by which nutraceutical agents promote longevity remain elusive.

Nectarine (*Prunus persica* var. nectarine) is a subspecies of peach that is grown and consumed worldwide [30]. Nectarine contains a number of nutritionally important health-promoting ingredients, including dietary fiber, meaningful amounts of vitamin C and β-carotenoids, and high contents of polyphenols [31–36]. A mouse study shows that both cytosolic and mitochondrial enzymatic activities in repairing 8-oxoguanine (8-oxoG) DNA lesions are significantly improved in aged mice fed a diet supplemented with a nectarine extract compared to age-matched control animals on a nonsupplemented diet [37]. Impairment of DNA repair capacities is linked to aging, cancer, and neurological diseases [38], and the mouse study supports the health benefits of nectarine consumption for its enhancement of DNA damage repair. However, there is no experimental evidence supporting any longevity-promoting property of nectarine at the organismic level.

Here we describe a series of studies designed to examine the effects of nectarine supplementation in the diet on life span in *Drosophila melanogaster*. *D. melanogaster* is an excellent model system to investigate the longevity-promoting properties of compounds and nutraceutical extracts because it has a short life span, can be cultured on simple diets, and has a rich genetic resource with a fully sequenced genome, and, more importantly, over half of the fly genes have mammalian homologs [39,40]. In this study, we demonstrate that nectarine can improve the survival of flies and that the extent of life span extension depends on gender, dietary conditions, and stress levels in flies.

Materials and methods

Materials and media

The cornmeal food for flies was made from cornmeal, sugar, yeast, and agar according to the published protocol [41]. The standard sugar–yeast extract (SY) diet contained 10% sugar, 10% yeast extract, and 1.5% agar. The high-fat diet was prepared by incorporating 2% palmitic acid (w/v) and 1% Tween 80 (v/v) into the standard SY diet as previously described [27]. The calorie-restricted diet contained 2.5% sugar, 2.5% yeast extract, and 1.5% agar. The freeze-dried nectarine extract was kindly provided by Paul Neipp at the USDA (Parlier, CA, USA) and stored at −80 °C until it was added to the standard, high-fat, and calorie-restricted diets at the final concentrations of 2, 4, and 8% (w/v).

Wild-type *D. melanogaster* strain *Canton S* was obtained from the Bloomington *Drosophila* Stock Center (Bloomington, IN, USA). Mutant flies with a *sod1* reduction of function were generated using the Gal4-UAS system combined with the RNA interference (RNAi) technique [42]. Specifically, double-stranded RNA of *sod1* was induced from a UAS-*sod1* inverted-repeat (UAS-*sod1IR*) line by a ubiquitously expressed Gal4, daughterless-Gal4 (da-Gal4). The UAS-*sod1IR* stock (strain F103) was originally generated by J. Phillips (University of Guelph, ON, Canada) [42]. Both UAS-*sod1IR* and da-Gal4 stocks were obtained from the Bloomington *Drosophila* Stock Center. All fly stocks were maintained on the cornmeal medium at 18 °C or room temperature before being used for various assays.

Life span, food intake, locomotor activity, and fecundity assays

To obtain adult flies for life span, food intake, locomotor activity, and fecundity assays, parental flies were allowed to mate and lay eggs on the cornmeal medium. After approximately 2 weeks, adult progeny flies of mixed sex within 24 h of eclosion were collected into new bottles with the standard SY medium. After mating for 24 h, males and females were sorted under light CO_2 anesthesia and placed separately into vials with approximately 5 ml of the standard SY medium. Each vial contained approximately 20 male or female flies. After another 24 h, flies were transferred to their respective treatment diets for life span, food intake, locomotor activity, and fecundity assays.

For the life span assay, flies were transferred to fresh food once every 2–3 days, and the number of dead flies was counted at each transfer. Life span data were recorded using Microsoft Excel (Microsoft, Redmond, WA, USA). Around 100–120 flies in five or six vials were included for each life span experiment. Each life span assay was repeated at least twice.

The capillary feeder method was employed to measure food intake as previously described [43]. Female flies were first treated with the standard, calorie-restricted, or high-fat diet supplemented with 0 or 4% nectarine for 11 days. Subsequently, eight flies were selected from each treatment and individually housed in the capillary feeding chamber. Each capillary was filled with the same food that each fly was fed before the feeding assay, except that the food in the capillaries contained no agar so that it would remain a liquid. Two capillaries with the food were set up in two separate feeding chambers without flies to account for evaporation of liquid food. Food intake was measured within 24 h. Final values of food intake were calculated by subtracting the evaporation and averaging food intake of six to eight live flies. Food intake was excluded for flies that died before the end of the experiment.

For the locomotor activity assay, spontaneous activity of 14-day-old females was measured after consumption of the standard, DR, or high-fat diet supplemented with or without 4% nectarine using the *Drosophila* Activity Monitor System (DAMS) from TriKinetics, Inc. (Waltham, MA, USA) according to the manufacturer's suggested protocol. The DAMS measures the number of times that a fly breaks the infrared beams by walking back and forth in a horizontal glass vial. Specifically, a glass vial with approximately 10 flies and the food supplemented with or without nectarine was inserted into the DAMS. The number of times that flies broke the infrared beams was recorded once every 20 s during a 24-h recording window. At the end of recording, the total number of surviving flies was recorded for each vial. Because the flies were undisturbed during the recording period, we operationally defined this activity as spontaneous locomotor activity. The 24-h spontaneous activity level was calculated by dividing the total number of beam breaks in 24 h by the total number of surviving flies in the vial. Recording spontaneous activities of females on each treatment was replicated five or six times with five or six vials, each with 10 females.

For the fecundity assay, after female flies were mated with males for 24 h, they were separated from males, placed on their respective diets, and then transferred to vials with fresh food once every 2–3 days. The number of dead flies was counted at each transfer.

The vials with old food and eggs on them were kept for egg counting. The number of eggs laid was counted and recorded in Microsoft Excel until all the flies were dead in a vial. Each egg-laying assay was repeated five or six times in separate vials. The lifetime fecundity was calculated by dividing the lifetime egg production in each vial by the number of females (approximately 10 flies per vial) at the beginning of the experiment.

Quantitative polymerase chain reaction (qPCR)

Three-day-old female flies were fed the standard SY, DR, or high-fat diet supplemented with 0, 2, or 4% nectarine until 14 days of age. Fly heads and bodies were frozen with liquid nitrogen and separated using a sieve. Total RNA was prepared from heads using the Trizol reagent from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's suggested protocol. Quality and quantity of total RNA were assessed using the Nanodrop 1000 from Thermo Scientific (Wilmington, DE, USA). cDNA was synthesized from the total RNA by using SuperScript reverse transcriptase from Invitrogen. qPCR was performed using the StepOnePlus real-time PCR system from Applied Biosystems (Foster City, CA, USA) according to the manufacturer's suggested protocol. The primer sequences for genes tested in this study were designed using the Primer3 program at http://primer3.sourceforge.net/ and are listed in Supplementary Table S2. Each qPCR measure was repeated three or four times with three or four biologically independent samples. Rp49 transcript was used as the reference for quantifying the relative transcript level of each selected gene.

Protein preparation

Mitochondrial and cytosolic proteins were prepared using the mitochondria isolation kit from MitoSciences (Cat. No. MS850). Briefly, approximately 40 14-day-old *Canton S* or *sod1RNAi* female flies fed the standard or high-fat diet supplemented with or without 4% nectarine extract were homogenized in ice-cold isolation buffer containing protein inhibitor cocktail (Roche Cat. No. 04693159001) using a glass Dounce homogenizer. Each homogenate was centrifuged at 1000*g* at 4 °C for 10 min. The supernatant was then transferred to a new tube and centrifuged again at 12,000*g* at 4 °C for 15 min to separate cytosolic and mitochondria proteins. The supernatants were collected as the cytosolic protein sample, and the pellet was washed and resuspended in 30 μl isolation buffer as the mitochondria protein sample. Protein concentration was determined with the BCA protein assay kit (Thermo Fisher, Cat. No. 23225) according to the manufacturer's suggested protocol.

Western blot analysis for 4-hydroxynonenal–protein adduct quantification

Protein samples (20 μg/lane) were separated by electrophoresis on a 7% NuPAGE Novex Tris–acetate SDS mini gel (Invitrogen, Cat. No. EA03585BOX) under reducing conditions according to the manufacturer's suggested protocol. After electrophoresis, proteins were transferred to a 0.2-μm polyvinylidene difluoride membrane for 7.5 min with the iBot Dry blotting system (Invitrogen, Cat. No. IB1001). Membrane-immobilized proteins were blocked with 5% nonfat dried milk in 1× Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 (TBST) for 1 h at room temperature and then incubated overnight at 4 °C with anti-N^{α}-acetyllysine-4-hydroxynonenal (4-HNE) fluorophore rabbit polyclonal primary antibody (Calbiochem, Cat. No. 393206) at a 1:4000 dilution in the blocking solution to detect 4-HNE–protein adducts [44]. The blots were washed three times (10 min each) with TBST and incubated in horseradish peroxidase-labeled goat anti-rabbit IgG diluted 1:3000 in the blocking solution for 90 min at room temperature. The immunoreactive proteins were detected with Amersham ECL Plus Western blotting detection reagents (GE Healthcare, Cat. No. RPN2132) and quantified using the ImageQuantTL software in the Typhoon Trio + imager system (GE Healthcare). The blots were then stripped and incubated with anti-β-actin antibody (Abcam, Cat. No. ab8224) and anti-complex I subunit NDUFS3 antibody (Mitosciences, Cat. No. MS112), both at a 1:2000 dilution to detect β-actin and NDUFS3 as the loading controls for cytosolic and mitochondrial proteins, respectively. For each protein sample, at least three biological replicates were used for immunostaining. For 4-HNE–protein adduct measurement, the protein region between molecular weights 460 and 268 kDa was quantified. Relative 4-HNE level was calculated by first normalizing the 4-HNE intensity value to that of β-actin and the ratios were adjusted again so that the ratio for the nonsupplemented control group was 1 for each comparison between supplemented and nonsupplemented groups.

Statistical analysis

All data were analyzed using StatView version 5.0 software (SAS, Cary, NC, USA). For the life span data, Mantel–Cox log rank tests were performed by comparing flies on the nectarine-supplemented diets to those on the nonsupplemented control diet. Maximum life span analysis was conducted on the longest-lived 10% of flies in each treatment. The two independent sets of life span data were analyzed either separately or by using data pooled from the two replicated experiments. For the spontaneous activity, food intake, fecundity, qPCR, and 4-HNE data, unpaired *t* tests were performed by comparing flies on the nectarine-supplemented diets to the nonsupplemented controls. The Bonferroni correction was conducted to adjust multiple comparisons. Statistical significance was set at $p \leq 0.05$. All mean and maximum life span, food intake, locomotion, fecundity, qPCR, and 4-HNE values are presented as means ± standard error. In addition, for expression of a gene to be considered significantly altered by supplementation with nectarine, the percentage of changes at the transcript level had to meet a stringent and operationally set level of ≥20%.

Results

The effects of nectarine extract on life span of flies fed a standard diet

Nectarine is rich in polyphenols and other antioxidants [32–34]. We hypothesized that nectarine may counteract oxidative damage and therefore extend the life span in flies. To test this hypothesis, we supplemented the standard SY diet for wild-type *Canton S* flies with 2 or 4% nectarine extract. No significant life span increase in male flies under the tested conditions was observed (Fig. 1A, Table 1 for results from combining two replicates, and Supplementary Tables S1 and S2 for replicated experiments). Based on the pooled data, supplementation with 4% nectarine slightly decreased mean and maximum life span of males fed the standard diet, but only by <8 and 2.5%, respectively ($p<0.01$, Table 1). Supplementation with 4% but not 2% nectarine significantly increased the mean life span of female flies by approximately 14–22% ($p<0.01$), but not consistently the maximum life span (Fig. 1B, Table 1, and Supplementary Tables S1 and S2). In one trial, supplementation with 2% nectarine decreased life span of females (Table S2). To determine whether food intake played a role in life span extension in female flies by nectarine, food intake within 24 h was measured. Supplementation with 4% nectarine did not significantly change the amount of food intake by females (Fig. 1C). These findings suggest that nectarine can promote longevity without affecting food intake, and this life span extension depends on gender and dosage of nectarine.

The effects of nectarine on life span of flies fed the DR diet

DR has been shown to extend life span in a wide variety of species [1]. To determine if nectarine and DR act on the same or different pathways, we measured the life span of *Canton S* flies fed the DR diet

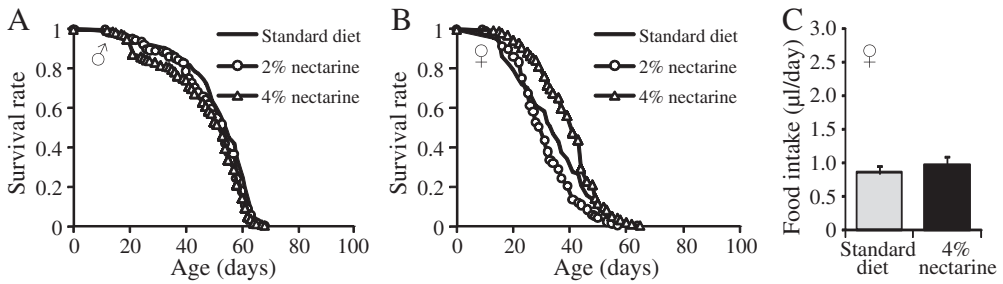

Fig. 1. The effects of nectarine on life span and food intake of flies fed the standard diet. (A) The graph shows life span of *Canton S* males fed the standard diet (10% sugar and 10% yeast extract) supplemented with no nectarine, which is labeled as Standard diet, or with 2 or 4% nectarine extract as labeled. More than 90 males were used in each life span assay. (B) The graph shows life span of *Canton S* females fed the standard diet supplemented with 0, 2, or 4% nectarine. More than 90 females were used in each life span assay. (C) Food intake was measured in 6–8 individually housed female flies fed the standard liquid diet supplemented with or without 4% nectarine. Food intake in microliters was calculated by averaging food intake of 6–8 flies within 24 h after subtracting the evaporation of the food. Error bars indicate standard errors. The life span curves were derived from two independent experiments.

supplemented with or without 2 or 4% nectarine. Supplementation with nectarine at both concentrations did not consistently affect the mean and maximum life span of males compared to the nonsupplemented controls (Fig. 2A, Table 1, and Supplementary Tables S1and S2). However, supplementation with nectarine at 2 or 4% significantly extended the mean life span of females by 7–11% and the maximum life span by 10–14% ($p<0.001$, Fig. 2B, Table 1, and Supplementary Tables S1and S2). No significant difference in food intake within 24 h was found between females fed the DR diet supplemented with or without 4% nectarine (Fig. 2C). These results suggest that the prolongevity effect of nectarine is mediated at least partly through DR-independent pathways.

The effects of nectarine on life span of flies fed a high-fat diet

Consumption of a high-fat diet is often associated with an increase in oxidative damage and a decrease in life span. To evaluate whether nectarine could promote the survival of flies fed a high-fat diet, we compared the life span of flies fed a high-fat diet supplemented with or without nectarine. Supplementation with 2 or 4% nectarine significantly increased the mean and maximum life span of males, but only marginally, by up to 5% in most cases compared to the nonsupplemented controls ($p<0.05$, Fig. 3A, Table 1, and Supplementary Tables S1 and S2). However, supplementation with nectarine at 8% did not extend the mean and maximum life span of males (Table 1). On the other hand, supplementation with nectarine at 2, 4, or 8% significantly extended both mean and maximum life span of females fed the high-fat diet by 10–20% ($p<0.01$, Fig. 3B, Table 1, and Supplementary Tables S1 and S2). Food intake was measured for females fed the high-fat diet supplemented with 0, 2, or 4% nectarine. Although there is a trend of slight decrease in food intake by nectarine, no statistically significant differences were found among the three groups of females ($p>0.1$, Fig. 3C). These findings suggest that nectarine can limit the adverse effects of the high-fat diet and promote the survival of flies on the high-fat diet.

Table 1
Life span of flies fed nectarine, combing two independent experiments.

Strain	Gender	Diet[a]	Total No. of flies	Mean life span (days ± SE)[b]	*p* for all flies[c]	No. of flies for max. life span	Max. life span (days ± SE)[b]	*p* for top 10% flies[c]
Canton S	Female	Standard	219	33.6 ± 0.9		22	54.6 ± 0.9	
Canton S	Female	Standard + 2% nect	215	31.1 ± 0.7	**0.002**	22	48.1 ± 1.5	**0.0091**
Canton S	Female	Standard + 4% nect	218	39.5 ± 0.7	**0.0003**	22	56.1 ± 0.9	0.3535
Canton S	Male	Standard	260	51.1 ± 0.8		26	64.5 ± 0.4	
Canton S	Male	Standard + 2% nect	222	49.3 ± 0.9	0.057	22	64.1 ± 0.4	0.3376
Canton S	Male	Standard + 4% nect	245	47.1 ± 0.9	**0.0002**	24	62.9 ± 0.4	**0.0042**
Canton S	Female	DR	213	53.1 ± 1.0		22	76.2 ± 0.9	
Canton S	Female	DR + 2% nect	216	57.3 ± 1.2	<**0.0001**	22	82.5 ± 1.2	**0.0002**
Canton S	Female	DR + 4% nect	205	64.7 ± 1.3	<**0.0001**	22	91.3 ± 0.9	<**0.0001**
Canton S	Male	DR	225	66.4 ± 0.8		22	79.0 ± 1.0	
Canton S	Male	DR + 2% nect	211	68.3 ± 0.7	**0.0532**	23	80.2 ± 1.1	0.2116
Canton S	Male	DR + 4% nect	238	66.8 ± 0.6	0.4731	24	78.5 ± 1.5	0.3262
Canton S	Female	High fat	244	30.5 ± 0.6		26	46.7 ± 0.7	
Canton S	Female	High fat + 2% nect	232	36.6 ± 0.7	<**0.0001**	24	52.3 ± 0.7	<**0.0001**
Canton S	Female	High fat + 4% nect	234	38.1 ± 0.8	<**0.0001**	24	59.5 ± 1.0	<**0.0001**
Canton S	Female	High fat + 8% nect	112	40.9 ± 0.9	<**0.0001**	12	54.6 ± 0.5	<**0.0001**
Canton S	Male	High fat	238	42.5 ± 0.9		25	59.4 ± 0.5	
Canton S	Male	High fat + 2% nect	235	46.8 ± 0.8	<**0.0001**	24	63.1 ± 0.4	<**0.0001**
Canton S	Male	High fat + 4% nect	240	45.2 ± 0.9	**0.0003**	25	62.4 ± 0.4	<**0.0001**
Canton S	Male	High fat + 8% nect	109	46.9 ± 1.0	0.1530	11	56.7 ± 0.3	<**0.0001**
sod1RNAi	Female	Standard	234	20.1 ± 0.3		24	26.8 ± 0.3	
sod1RNAi	Female	Standard + 2% nect	227	23.5 ± 0.3	<**0.0001**	23	31.7 ± 0.4	<**0.0001**
sod1RNAi	Female	Standard + 4% nect	226	25.9 ± 0.4	<**0.0001**	23	36.9 ± 0.8	<**0.0001**
sod1RNAi	Male	Standard	263	17.4 ± 0.3		27	27.3 ± 0.5	
sod1RNAi	Male	Standard + 2% nect	267	18.3 ± 0.3	0.0885	28	25.4 ± 0.6	0.0265
sod1RNAi	Male	Standard + 4% nect	265	17.9 ± 0.3	0.3865	27	25.3 ± 0.8	0.0316

[a] Standard, the standard diet containing 10% sugar and 10% yeast extract; nect, nectarine. The percentage value indicates the final concentration of nectarine in the food.
[b] Life span values are expressed as days ± standard error (SE).
[c] *p* values were calculated by comparing flies on the nectarine-supplemented diet to the corresponding nonsupplemented controls. Significant *p* values after the Bonferroni adjustment are in bold.

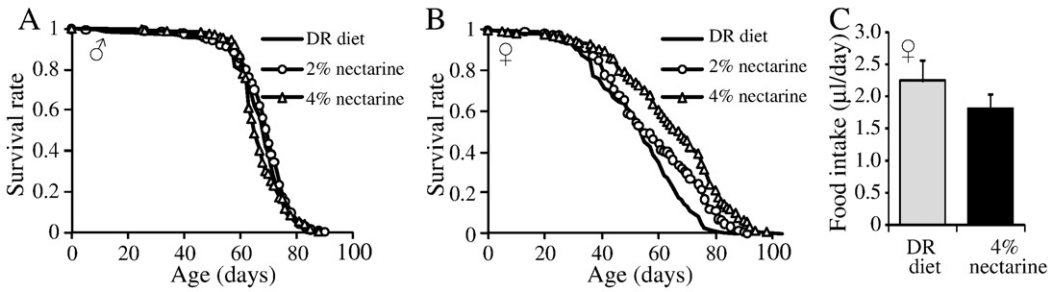

Fig. 2. The effects of nectarine on life span and food intake of flies fed the DR diet. (A) The graph shows life span of *Canton S* males fed the DR diet (2.5% sugar and 2.5% yeast extract) supplemented with no nectarine, which is labeled as DR diet, or with 2 or 4% nectarine extract as labeled. More than 90 males were used in each life span assay. (B) The graph shows life span of *Canton S* females fed the DR diet supplemented with 0, 2, or 4% nectarine. More than 90 females were used in each life span assay. (C) Food intake was measured in 6–8 individually housed female flies fed the DR liquid diet supplemented with or without 4% nectarine. No agar was added to the food in the capillaries. Food intake in microliters was calculated by averaging food intake of 6–8 flies within 24 h after subtracting the evaporation of the food. Error bars indicate standard errors. The life span curves were derived from two independent experiments.

The effects of nectarine on spontaneous activity of flies

Locomotor activity generally declines with age [45]. To test whether the life span extension by nectarine was associated with any changes in locomotor activity, we measured spontaneous locomotor activity of 14-day-old females fed the standard, DR, or high-fat diet supplemented with or without 4% nectarine. No obvious changes of the spontaneous locomotor activity were found between flies fed the diets supplemented with or without nectarine (Fig. 4A). These findings suggest that nectarine is not detrimental to the mobility of flies.

The effects of nectarine on fecundity

Life span extension in animals in some cases is associated with a decrease in reproduction [46,47]. To determine if the extension of life span by nectarine was associated with a decrease in reproduction, we determined the fecundity of female flies on the standard, DR, or high-fat diet supplemented with 0, 2, or 4% nectarine by measuring the egg production during the life of the female flies. The lifetime egg production by flies fed the diet supplemented with 2 or 4% nectarine was significantly increased compared to the nonsupplemented controls under all three dietary conditions (Fig. 4B). These findings indicate that supplementation with nectarine not only increases the life span but also maintains or even increases reproduction of female flies, suggesting that nectarine can delay reproductive aging.

The effects of nectarine on expression changes of genes associated with metabolism, oxidative stress, and longevity

To investigate the molecular mechanisms by which nectarine promotes life span and health span, we measured expression changes of genes associated with metabolism, oxidative stress, and longevity, including those in the insulin-like, JNK, sirtuin, and TOR signaling pathways, in heads of female flies fed the standard, DR, or high-fat diet supplemented with 0, 2, or 4% nectarine. The relative transcript levels of the genes tested in this study are listed in Supplementary Table S3 and expression patterns of some genes with significant changes induced by nectarine are depicted in Fig. 5.

For metabolism, a significant decrease in the transcript level of phosphoenolpyruvate carboxykinase (*PEPCK*), a key enzyme in gluconeogenesis, was found in flies fed the standard, DR, or high-fat diet supplemented with nectarine compared to the respective nonsupplemented controls. Iron regulatory protein 1B (*Irp-1B*), which is involved in iron metabolism, was down-regulated by nectarine in flies under all three dietary conditions.

For the oxidative stress response pathway, lethal (2) essential for life (*l(2)efl*) and heat shock protein 68 (*Hsp68*) were down-regulated, whereas metallothionein A (*MtnA*) was up-regulated by supplementation with 4% nectarine in flies fed the DR or high-fat diet but not the standard diet. These genes are downstream targets of the JNK pathway. However, the expression of other genes in the JNK pathway, including basket (*bsk*, the *Drosophila* JNK gene), puckered (*puc*), and glutathione *S*-transferase D1 (*GstD1*), was not changed by supplementation with nectarine. Among other stress response genes examined, mitochondrial superoxide dismutase 2 (*Sod2*) was down-regulated by nectarine in flies fed the high-fat diet, and catalase (*cat*) was down-regulated by nectarine in flies fed the standard diet, whereas female-specific independent of transformer (*fit*), which is induced under various stress conditions, was down-regulated by nectarine in flies fed the DR and high-fat diets.

Peroxiredoxins (Prx's) are a family of proteins with peroxidase activity and play an important role in oxidative stress response [48,49].

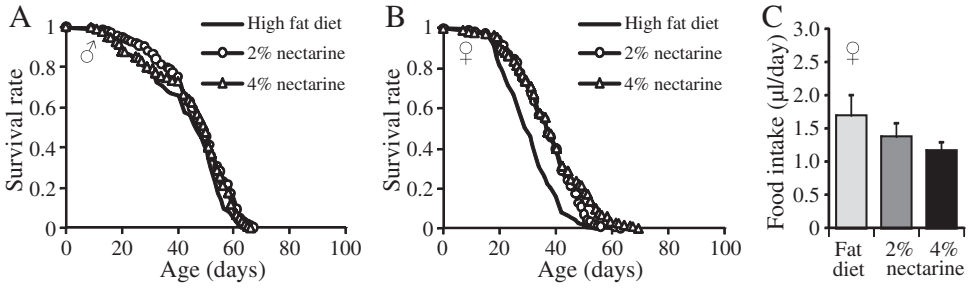

Fig. 3. The effects of nectarine on life span and food intake of flies fed the high-fat diet. (A) The graph shows life span of *Canton S* males fed the high-fat diet (10% sugar, 10% yeast extract, and 2% palmitic acid) supplemented with no nectarine, which is labeled as High fat diet, or with 2 or 4% nectarine as labeled. More than 90 males were used in each life span assay. (B) The graph shows life span of *Canton S* females fed the high-fat diet supplemented with 0, 2, or 4% nectarine. More than 90 females were used in each life span assay. (C) Food intake was measured in 6–8 individually housed female flies fed the high-fat liquid diet supplemented with or without 4% nectarine. Food intake in microliters was calculated by averaging food intake of 6–8 flies within 24 h after subtracting the evaporation of the food. Error bars indicate standard errors. The life span curves were derived from two independent experiments.

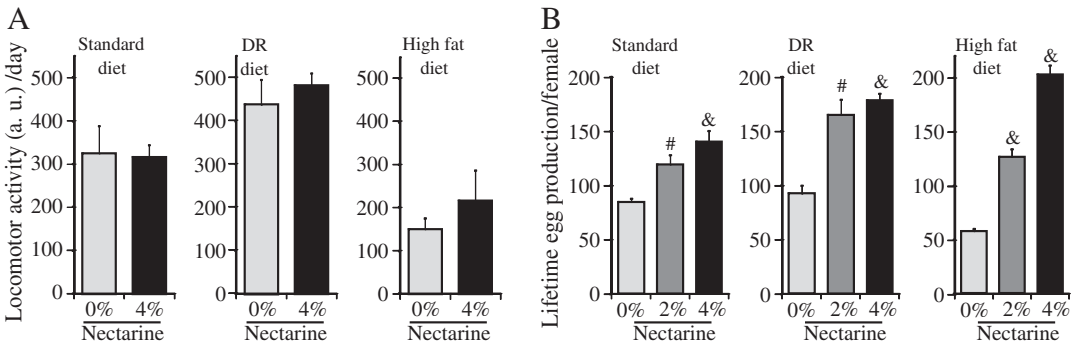

Fig. 4. The effects of nectarine on locomotor activity and fecundity of female flies. (A) Locomotor activity as spontaneous activity was measured for 14-day-old females fed the standard, DR, or high-fat diet supplemented with or without 4% nectarine. All females were pretreated for 11 days on the same diet on which the locomotion of the flies was measured. Error bars indicate standard errors. a.u. refers to arbitrary units. (B) Eggs laid by females were counted once every 2–3 days during the lifetime of approximately 10 female flies in each vial. The lifetime egg production was calculated by dividing the total lifetime egg production by the total number of females in each vial. Each egg production experiment was replicated five or six times. Error bars indicate standard errors. *p* values were calculated by comparing the nectarine-fed flies to the nonsupplemented controls. $^{\#}p<0.01$; $^{\&}p<0.001$.

Transcript levels of four Prx genes, *Prx2540, Prx5, Prx6005,* and *Prx5037,* were down-regulated by supplementation with 4% nectarine in flies fed the high-fat diet. In addition, *Prx6005* was down-regulated by nectarine in flies fed the standard but not the DR diet, whereas *Prx2540* was down-regulated by nectarine in flies fed the standard or the DR diet.

For the TOR signaling pathway, the transcript level of *4E-BP* but not *TOR* or *S6K* was significantly reduced by the supplementation with 4% nectarine in flies fed the standard or high-fat diet but not the DR diet. No significant alterations in gene expression were observed for genes involved in the insulin-like signaling pathway, including three *Drosophila* insulin-like peptides (*dllp2, dllp3,* and *dllp5*), insulin-like receptor substrate (*chico*), and forkhead transcription factor (*Foxo*), and in mitochondrial biogenesis, including nitric oxide synthase (*Nos*), mitochondrial assembly regulatory factor (*Marf*), and mitochondrial transcription factor A (*TFAM*) (Supplementary Table S3). Together, these findings indicate that supplementation with nectarine significantly influences the expression of genes involved in glucose metabolism, oxidative stress response, and detoxification.

The effects of nectarine on life span of sod1 mutant flies

To directly test whether the prolongevity effect of nectarine is related to antioxidative stress, we examined whether supplementation with nectarine could promote the survival of *sod1* mutant flies. Sod1 is a major cytosolic enzyme that scavenges highly reactive superoxides within the cell [50]. Loss- or reduction-of-function *sod1* mutants have higher amounts of oxidative damage to various macromolecules and shorter life span compared to wild-type flies [51]. We used the RNAi method to generate a reduction-of-function *sod1* mutant (*sod1RNAi*) and fed these flies the standard diet supplemented with 0, 2, or 4% nectarine. Similar to the findings in wild-type *Canton S* flies, supplementation with up to 4% nectarine did not consistently extend the mean and maximum life span of *sod1RNAi* males (Fig. 6A, Table 1, and

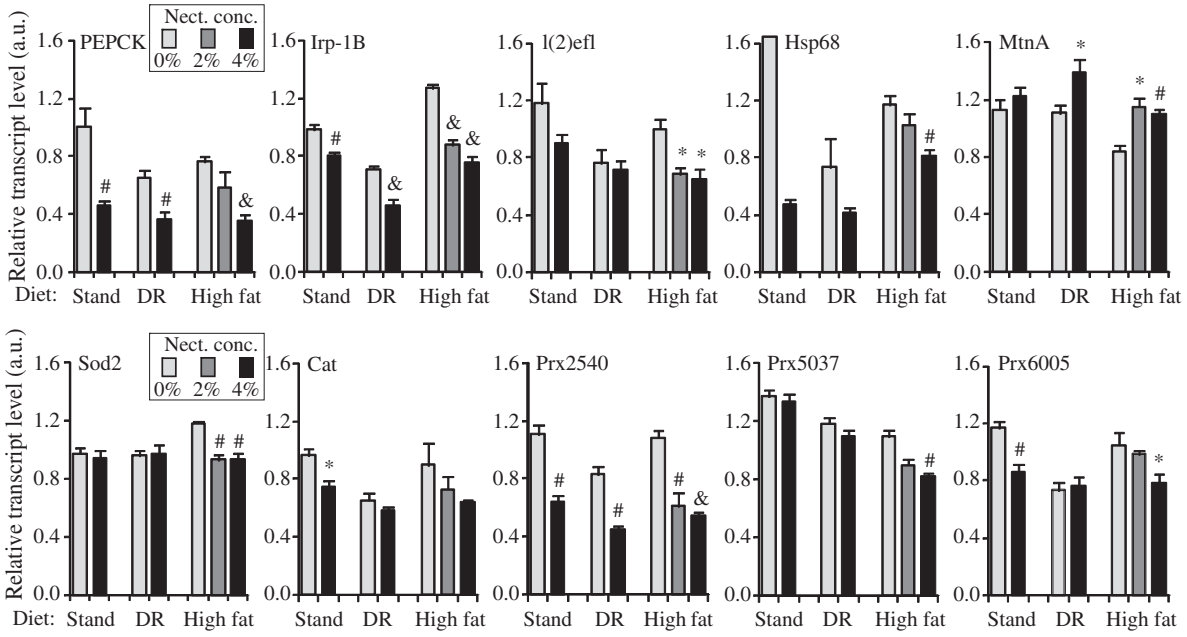

Fig. 5. The effect of nectarine on relative transcript levels of metabolism-, stress-, and longevity-associated genes. RNA was prepared from heads of 14-day-old female fruit flies after treatment on the standard, DR, or high-fat diet supplemented with or without nectarine for 11 days. Rp49 was used as the reference gene to calculate relative transcript levels of the tested genes. Gene names are shown at the upper left corner of each expression graph. Error bars indicate standard errors. *p* values were calculated by comparing the nectarine-fed flies to the nonsupplemented controls. a.u., arbitrary unit. $^{*}p\leq0.05$; $^{\#}p<0.01$; $^{\&}p<0.001$.

Supplementary Tables S1and S2). However, supplementation with nectarine at 2 or 4% significantly increased the mean and maximum life span of *sod1RNAi* females by 14–28% ($p<0.0001$, Fig. 6B, Table 1, and Supplementary Tables S1 and S2). Here the life span extension seems to be positively correlated with the concentration of nectarine. These results suggest that nectarine can alleviate the high level of oxidative damage and promote the survival in *sod1RNAi* female flies and also suggest that nectarine acts either downstream or independent of Sod1 to promote longevity.

The effects of nectarine on oxidative damage

To further examine the antioxidative stress property of nectarine, we determined the effect of nectarine on lipid oxidation as an indicator of oxidative damage by measuring the level of 4-HNE–protein adducts for wild-type *Canton S* or *sod1RNAi* female flies fed the standard SY or high-fat diet supplemented with or without 4% nectarine for 11 days. 4-HNE–protein adducts accumulate with age and under oxidative stress and are a commonly used biomarker of lipid oxidation [44,52,53]. We did not observe any significant change in the 4-HNE–protein adduct level in mitochondrial protein samples between any pair of diet-matched nectarine-supplemented and control female flies (data not shown). Supplementation with 4% nectarine did not induce any statistically significant change in the 4-HNE–protein adduct level in cytosolic protein samples for female flies on the standard SY diet compared to the nonsupplemented control, either ($p=0.20$, Fig. 7A). However, supplementation with 4% nectarine significantly reduced the 4-HNE–protein adduct level in the cytosolic protein samples by more than twofold for flies on the high-fat diet compared to the diet-matched nonsupplemented control ($p<0.01$, Fig. 7B). In addition, supplementation with 4% nectarine significantly reduced the 4-HNE–protein adduct level by approximately twofold for *sod1RNAi* female flies on the standard SY diet compared to the nonsupplemented control ($p<0.05$. Fig. 7C). These findings further support the notion that nectarine has antioxidative properties *in vivo*, at least for flies on the high-fat diet or oxidatively stressed *sod1RNAi* flies.

Discussion

Free radicals play an important role in aging and age-associated diseases [8,9]. Genetic mutations and environmental interventions that extend the life span of an organism are often associated with an increase in the resistance to oxidative stress in that organism. However, an alteration in oxidative stress response capacity alone does not necessarily lead to a change in life span, and the effects of oxidative stress response pathways on life span and aging depend on the environmental context and genetic background of an organism [9]. Nevertheless, reducing oxidative damage remains one of the promising interventions to delay the progression of aging and age-related diseases. Polyphenols are a family of phytochemicals possessing high antioxidant capacities and other health-promoting bioactivities, such as activators of enzymes and ligand mimetics of receptors [4,5,19]. Many fruits contain significant amounts of polyphenols and, therefore, are ideal candidates for prolongevity interventions. However, evidence directly demonstrating the prolongevity properties of fruits is scarce. In this study, we have demonstrated that an extract from nectarine can promote longevity in female flies under various dietary conditions, including the standard, DR, and high-fat diets. Supplementation with nectarine can also promote the survival of flies with high levels of oxidative damage resulting from a reduction-of-function mutation in *sod1*, a major superoxide scavenger in the cell [50]. In addition, nectarine can increase fecundity and duration of egg production (data not shown), which suggests that nectarine can delay reproductive aging. Furthermore, qPCR and Western blot analyses suggest that nectarine promotes longevity at least partly through reducing oxidative damage. This study is the first demonstrating the prolongevity property of nectarine, a globally grown and consumed fruit.

Composition of dietary nutrients plays an important role in modulating life span [46,54,55]. DR has been shown to extend the life span in diverse species, although the genetic background of an organism also contributes to the DR life span extension effect [1,17,18]. Consumption of high-fat diets induces higher levels of oxidative stress and results in shorter life span compared to low-fat diets [20,56]. In the studies reported here, we have investigated how supplementation with nectarine affects the life span in flies fed three different diets, standard, DR, and high fat. Supplementation with 4% nectarine is sufficient to extend life span of female flies under all the dietary conditions. Supplementation with 2% nectarine consistently extends lifespan only of females on the high-fat diet. However, supplementation with nectarine up to 4% only marginally extends life span in males fed the high-fat diet but not the standard or DR diet compared to the respective nonsupplemented controls. It is unclear why nectarine is much more effective in promoting longevity in females than in males. Further study is needed to determine whether the differences in physiology, such as the energy requirement for reproduction, contribute to the gender-specific response [57]. Nevertheless, our findings suggest that the extent of life span extension by nectarine in flies depends on gender, dosage of nectarine, and nutrient composition of the diet. This is consistent with previous observations using polyphenols and fruit extracts, e.g., the prolongevity effect of resveratrol in some organisms seems to depend on the dietary conditions [20,22,23,58]. Resveratrol has been shown to promote the survival of mice on a high-fat diet but not on the standard

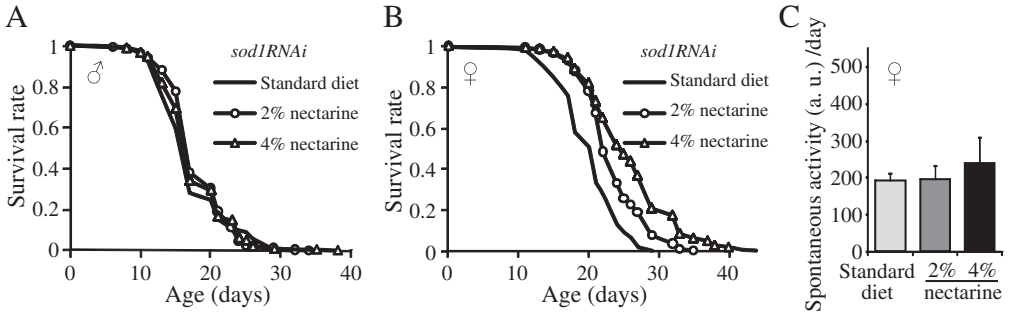

Fig. 6. The effects of nectarine on the life span and locomotor activity of *sod1RNAi* flies fed the standard diet. (A) The graph shows life span of *sod1RNAi* males fed the standard diet (10% sugar and 10% yeast extract) supplemented with no nectarine, which is labeled as Standard diet, or with 2 or 4% nectarine as labeled. More than 90 males were used in each life span assay. (B) The graph shows life span of *sod1RNAi* females fed the standard diet supplemented with 0, 2, or 4% nectarine. More than 90 females were used in each life span assay. (C). Locomotor activity was measured for 14-day-old *sod1RNAi* females fed the standard liquid diet supplemented with 0, 2, or 4% nectarine. All flies were pretreated for 11 days on the same diet on which locomotion activity of flies was measured. Error bars indicate standard errors. a.u., arbitrary unit. The life span curves were derived from two independent experiments.

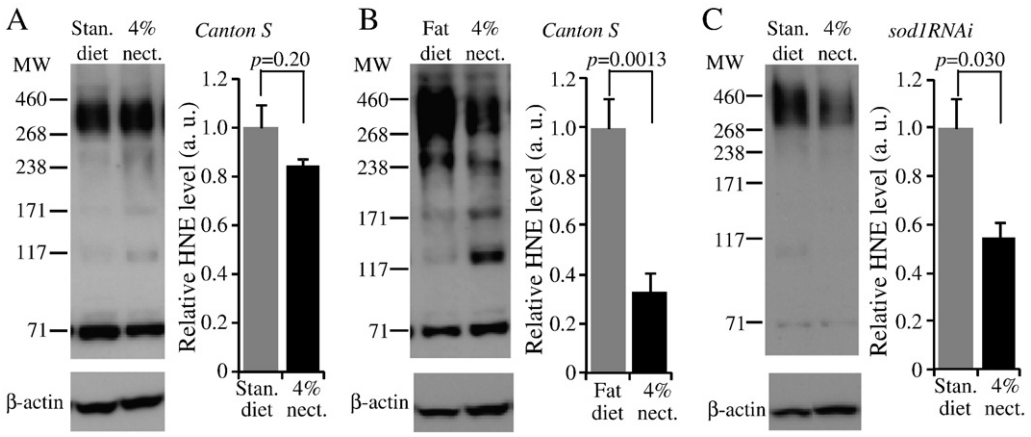

Fig. 7. The effects of nectarine on lipid oxidation. Lipid oxidation was determined by measuring the level of 4-HNE–protein adducts. Representative Western blot images for cytosolic proteins are shown. Intensity in the area between molecular weights (MW) 460 and 268 kDa was measured as the 4-HNE–protein adduct level of flies in each treatment. β-Actin was used as protein loading control to normalize the 4-HNE–protein adduct level. Relative 4-HNE levels were calculated from three to five biologically independent samples for each treatment and are shown as bar graphs on the right side of the gel images. For each comparison, the average 4-HNE level of the nonsupplemented group was adjusted to the value 1. (A) *Canton S* female flies were fed the standard SY diet supplemented with or without 4% nectarine (biological replicates, $n=3$). (B) *Canton S* female flies were fed the high-fat diet with or without 4% nectarine ($n=5$). (C) *sod1RNAi* female flies were fed the standard SY diet supplemented with or without 4% nectarine ($n=3$).

rodent diet [20]. Furthermore, resveratrol extends the life span in female Mexflies only when on a restricted and high-sugar diet [58]. In addition, a mixture of cranberry and oregano extracts can promote longevity in the Mexfly on high-sugar diets but not high-protein diets [26]. Açai pulp extract has been shown to improve the survival of female flies on a high-fat but not a standard fly diet [27]. Collectively, these findings indicate that the dietary conditions are critical for the prolongevity effects of nutraceutical agents and that the life span extension by different fruits may be mediated by different pathways.

Aging is associated with a decline in reproductive capacity [59]. We have found that supplementation with 2 or 4% nectarine under all dietary conditions significantly increases lifetime fecundity in females without an obvious change in food intake in these flies compared to the nonsupplemented flies. Considering the calorie and protein contents of a typical nectarine [35], addition of 2% nectarine into the high-fat diet with 10% sugar, 10% yeast extract, and 2% palmitic acid results in <6% extra calories and <2% extra proteins compared to the non-supplemented diet. However, the difference in lifetime egg production between flies on the high-fat diets supplemented with and without nectarine is more than twofold. Therefore, it is unlikely that the difference in lifetime fecundity is mainly due to the differences in the calorie and protein contents in the food. It is possible that nectarine improves general health of animals, maintains health of reproductive organs, such as the ovary, or even directly regulates reproduction as a hormone mimetic and consequently extends the reproductive capacity and health span. Further molecular, cellular, and genetic studies should reveal the mechanisms by which nectarine delays reproductive aging.

A number of genetic pathways have been shown to modulate life span in yeast, worms, flies, and rodents [1,2], and many genes are altered at the transcription level with age [60,61]. Some of these genes mediate the prolongevity effect of DR, and some are linked to human longevity. We have assessed whether nectarine promotes longevity through the DR pathways and have found that nectarine can further extend the life span of flies on the DR diet. This suggests that the prolongevity effect of nectarine is at least partly mediated through DR-independent pathways. To determine the relationship between nectarine and longevity-associated pathways, we have surveyed the expression of a number of genes involved in various biological processes, including glucose metabolism, oxidative stress response, and protein synthesis. Two prominent features emerge from this survey. One is that nectarine reduces the expression of PEPCK, a key enzyme in gluconeogenesis, in flies fed all three diets [62]. We have previously observed a decrease in PEPCK expression by supplementation with açai pulp extract in flies fed the high-fat diet. Inhibition of PEPCK activity by the drug fenofibrate is one of the therapeutic approaches to manage type 2 diabetes [63]. Although gluconeogenesis and the glucose level were not measured in our studies, our findings suggest that fruits rich in polyphenols have the potential to modulate glucose metabolism in a health-promoting manner.

The second finding revealed by gene expression analysis is that genes involved in the oxidative stress-response pathways tend to be down-regulated by nectarine. The extent of down-regulation depends on the dietary conditions. Nectarine decreases expression of two heat shock proteins, *l(2)efl* and *Hsp68,* but increases expression of *MtnA* in flies fed the high-fat diet. These three genes are downstream targets of the JNK signaling pathway and are up-regulated in response to oxidative stress [64,65]. Interestingly, açai pulp extract has been shown to increase expression of these three genes in flies fed the high-fat diet. How nectarine differentially modulates expression of the JNK target genes is unclear. The JNK pathway has been implicated in modulating the life span in *D. melanogaster* [64,65]. We postulate that nectarine indirectly modulates the JNK signaling pathway by influencing the redox status and reducing oxidative damage in the cell, whereas açai may directly activate the JNK pathway.

Our hypothesis is further supported by expression patterns of three Prx's, *Prx2540, Prx6005,* and *Prx5037,* a family of antioxidant enzymes involved in detoxifying peroxide in the cell [48,66]. Nectarine decreases expression of all three Prx's in flies fed the high-fat diet. Furthermore, nectarine decreases expression of *Prx2540* in flies fed the standard or DR diet and reduces the transcript level of *Prx6005* in flies fed the DR diet. Prx's tend to be up-regulated by increased oxidative stress. Consistent with the expression patterns of the JNK downstream genes, decreased expression of Prx's by supplementation with nectarine seems to be indirectly mediated by the effect of nectarine to reduce oxidative damage and/or improve the redox status. Furthermore, nectarine significantly reduces lipid oxidation as indicated by the 4-HNE–protein adduct level in cytosolic proteins of wild-type female flies fed the high-fat diet and *sod1RNAi* female flies fed the standard diet compared to the respective nonsupplemented controls. These results are also consistent with observations made in mice fed nectarine. Mice fed nectarine have a higher 8-oxoG DNA lesion repair capacity than the nonsupplemented controls [37]. Several genes involved in detoxification, including *Sod1,* glutathione peroxidase 1, and glutathione *S*-transferase Alpha 3 (*GSTA3*), have been found to be down-regulated in 18- to 19-month-old mice fed a nectarine-supplemented diet for 14–16 weeks compared to nonsupplemented controls [37]. Although nectarine does not necessarily reduce

expression of the fly genes homologous to these mouse genes, these observations suggest that down-regulation of genes involved in detoxification is a conserved property of nectarine. Furthermore, both nectarine and açai significantly extend the life span of *sod1RNAi* female flies with high levels of oxidative damage despite the difference in modulating expression of stress-response genes. Taken together, these findings suggest that functional fruits such as nectarine and açai can promote survival by reducing oxidative damage and/or activating stress-response signaling pathways.

Consumption of fruits can provide numerous health benefits [5]. However, development of effective and relatively low-cost prolongevity interventions with functional fruits is still in the early stage. A survey conducted in five European countries indicates that Europeans consume two to four nectarines and peaches per week on average [30]. Nectarine provides valuable macronutrients and micronutrients. An analysis on several nectarine cultivars in Australia has shown that the typical weight of a nectarine is approximately 90 g and over 90% of it is edible, with total energy content at approximately 1.6 kJ/g edible portion [35]. Among the edible part, more than 80% is water, approximately 7.5% is sugar, 2% is dietary fiber, and 1% is protein [35]. More importantly, nectarine contains high contents of β-carotene and vitamin C and meaningful amounts of polyphenols in forms both extractable by organic solvents and nonextractable but hydrolyzable, associated with dietary fiber and protein [31,33,34]. Our study indicates that supplementation of food with 4% nectarine is sufficient to promote life span and health span in the fly. A recent mouse study indicates that supplementation of food with 8% nectarine can induce a higher level of DNA repair activity [37]. These dosages of nectarine correspond to five to eight nectarines per day for an average adult human [37]. Our study in *Drosophila* is the first to show life span extension by nectarine in an animal model and thus provides a foundation for future research and development of nectarine as an effective intervention to promote life span and health span in mammals, including humans.

Supplementary materials related to this article can be found online at doi:10.1016/j.freeradbiomed.2011.03.011.

Acknowledgments

We thank Paul Neipp for preparing the nectarine extract, Dr. Vilhelm Bohr for encouraging us to initiate this study, and Dr. Nan-ping Weng and Edward Spangler for valuable comments and suggestions on the manuscript. This study was supported by the Intramural Research Program of the National Institute on Aging, NIH, to S.Z.

References

[1] Fontana, L.; Partridge, L.; Longo, V. D. Extending healthy life span—from yeast to humans. *Science* **328:**321–326; 2010.
[2] Kenyon, C. J. The genetics of ageing. *Nature* **464:**504–512; 2010.
[3] Bao, Y.; Fenwick, R. Phytochemicals in Health and Disease. Dekker, New York; 2004.
[4] Liu, R. H. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutr.* **134:**3479S–3485S; 2004.
[5] Joseph, J. A.; Shukitt-Hale, B.; Lau, F. C. Fruit polyphenols and their effects on neuronal signaling and behavior in senescence. *Ann. N. Y. Acad. Sci.* **1100:**470–485; 2007.
[6] Pan, M. H.; Lai, C. S.; Dushenkov, S.; Ho, C. T. Modulation of inflammatory genes by natural dietary bioactive compounds. *J. Agric. Food Chem.* **57:**4467–4477; 2009.
[7] Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11:**298–300; 1956.
[8] Muller, F. L.; Lustgarten, M. S.; Jang, Y.; Richardson, A.; Van Remmen, H. Trends in oxidative aging theories. *Free Radic. Biol. Med.* **43:**477–503; 2007.
[9] Perez, V. I.; Bokov, A.; Remmen, H. V.; Mele, J.; Ran, Q.; Ikeno, Y.; Richardson, A. Is the oxidative stress theory of aging dead? *Biochim. Biophys. Acta* **1790:**1005–1014; 2009.
[10] Guarente, L. Sirtuins in aging and disease. *Cold Spring Harb. Symp. Quant. Biol.* **72:** 483–488; 2007.
[11] Allard, J. S.; Perez, E.; Zou, S.; de Cabo, R. Dietary activators of Sirt1. *Mol. Cell. Endocrinol.* **299:**58–63; 2009.
[12] Barbieri, M.; Rizzo, M. R.; Papa, M.; Boccardi, V.; Esposito, A.; White, M. F.; Paolisso, G. The IRS2 Gly1057Asp variant is associated with human longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **65:**282–286; 2010.
[13] Barzilai, N.; Bartke, A. Biological approaches to mechanistically understand the healthy life span extension achieved by calorie restriction and modulation of hormones. *J. Gerontol. A Biol. Sci. Med. Sci.* **64:**187–191; 2009.
[14] Capri, M.; Salvioli, S.; Sevini, F.; Valensin, S.; Celani, L.; Monti, D.; Pawelec, G.; De Benedictis, G.; Gonos, E. S.; Franceschi, C. The genetics of human longevity. *Ann. N. Y. Acad. Sci.* **1067:**252–263; 2006.
[15] Suh, Y.; Atzmon, G.; Cho, M. O.; Hwang, D.; Liu, B.; Leahy, D. J.; Barzilai, N.; Cohen, P. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl Acad. Sci. U. S. A.* **105:**3438–3442; 2008.
[16] Masoro, E. J. Dietary restriction-induced life extension: a broadly based biological phenomenon. *Biogerontology* **7:**153–155; 2006.
[17] Liao, C. Y.; Rikke, B. A.; Johnson, T. E.; Diaz, V.; Nelson, J. F. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell* **9:**92–95; 2010.
[18] Sohal, R. S.; Ferguson, M.; Sohal, B. H.; Forster, M. J. Life span extension in mice by food restriction depends on an energy imbalance. *J. Nutr.* **139:**533–539; 2009.
[19] Sies, H. Polyphenols and health: update and perspectives. *Arch. Biochem. Biophys.* **501:**2–5; 2010.
[20] Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444:**337–342; 2006.
[21] Pearson, K. J.; Baur, J. A.; Lewis, K. N.; Peshkin, L.; Price, N. L.; Labinskyy, N.; Swindell, W. R.; Kamara, D.; Minor, R. K.; Perez, E.; Jamieson, H. A.; Zhang, Y.; Dunn, S. R.; Sharma, K.; Pleshko, N.; Woollett, L. A.; Csiszar, A.; Ikeno, Y.; Le Couteur, D.; Elliott, P. J.; Becker, K. G.; Navas, P.; Ingram, D. K.; Wolf, N. S.; Ungvari, Z.; Sinclair, D. A.; de Cabo, R. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* **8:**157–168; 2008.
[22] Valenzano, D. R.; Terzibasi, E.; Genade, T.; Cattaneo, A.; Domenici, L.; Cellerino, A. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* **16:**296–300; 2006.
[23] Wood, J. G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S. L.; Tatar, M.; Sinclair, D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430:**686–689; 2004.
[24] Pirola, L.; Frojdo, S. Resveratrol: one molecule, many targets. *IUBMB Life* **60:** 323–332; 2008.
[25] Wilson, M. A.; Shukitt-Hale, B.; Kalt, W.; Ingram, D. K.; Joseph, J. A.; Wolkow, C. A. Blueberry polyphenols increase lifespan and thermotolerance in Caenorhabditis elegans. *Aging Cell* **5:**59–68; 2006.
[26] Zou, S.; Carey, J. R.; Liedo, P.; Ingram, D. K.; Yu, B.; Ghaedian, R. Prolongevity effects of an oregano and cranberry extract are diet dependent in the Mexican fruit fly (Anastrepha ludens). *J. Gerontol. A Biol. Sci. Med. Sci.* **65:**41–50; 2010.
[27] Sun, X.; Seeberger, J.; Alberico, T.; Wang, C.; Wheeler, C. T.; Schauss, A. G.; Zou, S. Acai palm fruit (Euterpe oleracea Mart.) pulp improves survival of flies on a high fat diet. *Exp. Gerontol.* **45:**243–251; 2010.
[28] Bass, T. M.; Weinkove, D.; Houthoofd, K.; Gems, D.; Partridge, L. Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. *Mech. Ageing Dev.* **128:**546–552; 2007.
[29] Kaeberlein, M.; Kennedy, B. K. Does resveratrol activate yeast Sir2 in vivo? *Aging Cell* **6:**415–416; 2007.
[30] Konopacka, D.; Jesionkowska, K.; Kruczynska, D.; Stehr, R.; Schoorl, F.; Buehler, A.; Egger, S.; Codarin, S.; Hilaire, C.; Holler, I.; Guerra, W.; Liverani, A.; Donati, F.; Sansavini, S.; Martinelli, A.; Petiot, C.; Carbo, J.; Echeverria, G.; Iglesias, I.; Bonany, J. Apple and peach consumption habits across European countries. *Appetite* **55:** 478–483; 2010.
[31] Arranz, S.; Saura-Calixto, F.; Shaha, S.; Kroon, P. A. High contents of nonextractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. *J. Agric. Food Chem.* **57:**7298–7303; 2009.
[32] Cantin, C. M.; Moreno, M. A.; Gogorcena, Y. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [Prunus persica (L.) Batsch] breeding progenies. *J. Agric. Food Chem.* **57:**4586–4592; 2009.
[33] Gil, M. I.; Tomas-Barberan, F. A.; Hess-Pierce, B.; Kader, A. A. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* **50:**4976–4982; 2002.
[34] Tomas-Barberan, F. A.; Gil, M. I.; Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A. A. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J. Agric. Food Chem.* **49:**4748–4760; 2001.
[35] Wills, R. B.; Scriven, F. M.; Greenfield, H. Nutrient composition of stone fruit (Prunus spp.) cultivars: apricot, cherry, nectarine, peach and plum. *J. Sci. Food Agric.* **34:**1383–1389; 1983.
[36] Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* **49:**5315–5321; 2001.
[37] Croteau, D. L.; de Souza-Pinto, N. C.; Harboe, C.; Keijzers, G.; Zhang, Y.; Becker, K.; Sheng, S.; Bohr, V. A. DNA repair and the accumulation of oxidatively damaged DNA are affected by fruit intake in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **65:** 1300–1311; 2010.
[38] Wilson III, D. M.; Bohr, V. A.; McKinnon, P. J. DNA damage, DNA repair, ageing and age-related disease. *Mech. Ageing Dev.* **129:**349–352; 2008.
[39] M.A. Jones, M. Grotewiel, Drosophila as a model for age-related impairment in locomotor and other behaviors. Exp. Gerontol. 2010, doi:10.1016/j.exger.2010.08.012. [Electronic publication ahead of print].
[40] Zou, S.; Sinclair, J.; Wilson, M. A.; Carey, J. R.; Liedo, P.; Oropeza, A.; Kalra, A.; de Cabo, R.; Ingram, D. K.; Longo, D. L.; Wolkow, C. A. Comparative approaches

to facilitate the discovery of prolongevity interventions: effects of tocopherols on lifespan of three invertebrate species. *Mech. Ageing Dev.* **128:**222–226; 2007.
[41] Ashburner, M., Golic, K.G., Hawley, R.S. (Eds.), 2005]. Drosophila: a Laboratory Handbook. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; 2005.
[42] Missirlis, F.; Hu, J.; Kirby, K.; Hilliker, A. J.; Rouault, T. A.; Phillips, J. P. Compartment-specific protection of iron–sulfur proteins by superoxide dismutase. *J. Biol. Chem.* **278:**47365–47369; 2003.
[43] Ja, W. W.; Carvalho, G. B.; Mak, E. M.; de la Rosa, N. N.; Fang, A. Y.; Liong, J. C.; Brummel, T.; Benzer, S. Prandiology of Drosophila and the CAFE assay. *Proc. Natl Acad. Sci. U. S. A.* **104:**8253–8256; 2007.
[44] Tsai, L.; Szweda, P. A.; Vinogradova, O.; Szweda, L. I. Structural characterization and immunochemical detection of a fluorophore derived from 4-hydroxy-2-nonenal and lysine. *Proc. Natl Acad. Sci. U. S. A.* **95:**7975–7980; 1998.
[45] Grotewiel, M. S.; Martin, I.; Bhandari, P.; Cook-Wiens, E. Functional senescence in Drosophila melanogaster. *Ageing Res. Rev.* **4:**372–397; 2005.
[46] Carey, J. R.; Harshman, L. G.; Liedo, P.; Muller, H. G.; Wang, J. L.; Zhang, Z. Longevity–fertility trade-offs in the tephritid fruit fly, Anastrepha ludens, across dietary-restriction gradients. *Aging Cell* **7:**470–477; 2008.
[47] Carey, J. R.; Molleman, F. Reproductive aging in tephritid fruit flies. *Ann. N. Y. Acad. Sci.* **1204:**139–148; 2010.
[48] Radyuk, S. N.; Klichko, V. I.; Spinola, B.; Sohal, R. S.; Orr, W. C. The peroxiredoxin gene family in *Drosophila melanogaster*. *Free Radic. Biol. Med.* **31:**1090–1100; 2001.
[49] Wood, Z. A.; Poole, L. B.; Karplus, P. A. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* **300:**650–653; 2003.
[50] Fridovich, I. Superoxide dismutases. *Annu. Rev. Biochem.* **44:**147–159; 1975.
[51] Kirby, K.; Hu, J.; Hilliker, A. J.; Phillips, J. P. RNA interference-mediated silencing of Sod2 in Drosophila leads to early adult-onset mortality and elevated endogenous oxidative stress. *Proc. Natl Acad. Sci. U. S. A.* **99:**16162–16167; 2002.
[52] Ferrara, N.; Rinaldi, B.; Corbi, G.; Conti, V.; Stiuso, P.; Boccuti, S.; Rengo, G.; Rossi, F.; Filippelli, A. Exercise training promotes SIRT1 activity in aged rats. *Rejuvenation Res.* **11:**139–150; 2008.
[53] Patel, V. B.; Spencer, C. H.; Young, T. A.; Lively, M. O.; Cunningham, C. C. Effects of 4-hydroxynonenal on mitochondrial 3-hydroxy-3-methylglutaryl (HMG-CoA) synthase. *Free Radic. Biol. Med.* **43:**1499–1507; 2007.
[54] Lee, K. P.; Simpson, S. J.; Clissold, F. J.; Brooks, R.; Ballard, J. W.; Taylor, P. W.; Soran, N.; Raubenheimer, D. Lifespan and reproduction in Drosophila: new insights from nutritional geometry. *Proc. Natl Acad. Sci. U. S. A.* **105:**2498–2503; 2008.
[55] Skorupa, D. A.; Dervisefendic, A.; Zwiener, J.; Pletcher, S. D. Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. *Aging Cell* **7:**478–490; 2008.
[56] Driver, C. J.; Wallis, R.; Cosopodiotis, G.; Ettershank, G. Is a fat metabolite the major diet dependent accelerator of aging? *Exp. Gerontol.* **21:**497–507; 1986.
[57] Tower, J. Sex-specific regulation of aging and apoptosis. *Mech. Ageing Dev.* **127:** 705–718; 2006.
[58] Zou, S.; Carey, J. R.; Liedo, P.; Ingram, D. K.; Muller, H. G.; Wang, J. L.; Yao, F.; Yu, B.; Zhou, A. The prolongevity effect of resveratrol depends on dietary composition and calorie intake in a tephritid fruit fly. *Exp. Gerontol.* **44:**472–476; 2009.
[59] Haaga, J.; O'Connor, K.; Weinstein, M.; Wise, P. Reproductive aging: theoretical perspectives, mechanisms, nonhuman models, and health correlates. *Ann. N. Y. Acad. Sci.* **1204:**1–10; 2010.
[60] Lu, T.; Pan, Y.; Kao, S. Y.; Li, C.; Kohane, I.; Chan, J.; Yankner, B. A. Gene regulation and DNA damage in the ageing human brain. *Nature* **429:**883–891; 2004.
[61] Zhan, M.; Yamaza, H.; Sun, Y.; Sinclair, J.; Li, H.; Zou, S. Temporal and spatial transcriptional profiles of aging in Drosophila melanogaster. *Genome Res.* **17:** 1236–1243; 2007.
[62] Chakravarty, K.; Cassuto, H.; Reshef, L.; Hanson, R. W. Factors that control the tissue-specific transcription of the gene for phosphoenolpyruvate carboxykinase-C. *Crit. Rev. Biochem. Mol. Biol.* **40:**129–154; 2005.
[63] Srivastava, R. A. Fenofibrate ameliorates diabetic and dyslipidemic profiles in KKAy mice partly via down-regulation of 11beta-HSD1, PEPCK and DGAT2: comparison of PPARalpha, PPARgamma, and liver X receptor agonists. *Eur. J. Pharmacol.* **607:**258–263; 2009.
[64] Karpac, J.; Jasper, H. Insulin and JNK: optimizing metabolic homeostasis and lifespan. *Trends Endocrinol. Metab.* **20:**100–106; 2009.
[65] Wang, M. C.; Bohmann, D.; Jasper, H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* **121:** 115–125; 2005.
[66] Radyuk, S. N.; Sohal, R. S.; Orr, W. C. Thioredoxin peroxidases can foster cytoprotection or cell death in response to different stressors: over- and under-expression of thioredoxin peroxidase in Drosophila cells. *Biochem. J.* **371:**743–752; 2003.